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ACETYLDEHYDRORISHITINOL, A RISHITINOL-RELATED POTATO STRESS METABOLITE

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Key Word Index—*Solanum tuberosum*; Solanaceae; white potato cv. Katahdin; sesquiterpenoid stress metabolites; effect of ethylene/oxygen; phytoalexins; rishitinol; acetyldehydrorishitinol.

Abstract—A controlled atmosphere of ethylene in oxygen elevates rishitin and phytuberin levels in hypersensitive potato tuber tissue and the levels of hitherto unidentified stress metabolites. One of these was isolated and mass spectral, NMR, IR and UV data suggest the structure, 5,8-dimethyl-2[2 acetoxyisopropyl]-1,4-dihydronaphthalene. This new stress metabolite is related to rishitinol by loss of water and acetylation.

INTRODUCTION

The production of sesquiterpenoid stress metabolites (SSMs) by tuber tissue is characteristic of the hypersensitive response of certain varieties of the white potato (*Solanum tuberosum* L.) to strains of the late blight fungus *Phytophthora infestans* (Mont.) de Bary [1]. Four of the major SSMs produced by *S. tuberosum*, katahdinone (solavetivone), lubimin, rishitin and phytuberin, possess fungistatic or fungitoxic properties [2]. The inhibition of fungal growth in hypersensitive interactions has been attributed to the presence of these SSMs in host tuber tissue, and for this reason they are sometimes termed phytoalexins [3].

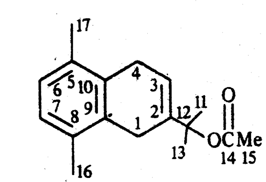
Production of SSMs can be elicited in uninfected tuber tissue by treatment with a cell-free extract of *P. infestans* [4] and we have previously reported that incubation in an ethylene/oxygen controlled atmosphere greatly elevates levels of both known and unidentified stress metabolites in such tissues [5, 6]. At least four unidentified compounds reacting to $SbCl_3$ and having different R_fs were observed on TLC plates of methanol extracts from hypersensitive tuber tissues. These stress metabolites were present in very low concentration as judged by TLC staining intensity in tuber tissue incubated in air. Here we report the identification of one of the unknowns as 5,8-dimethyl-2[2 acetoxyisopropyl]-1,4-dihydronaphthalene.

RESULTS AND DISCUSSION

The UV absorption maximum of 5,8-dimethyl-2[2 acetoxyisopropyl]-1,4-dihydronaphthalene (**1**) at 268 nm indicates the presence of an aromatic ring (cf rishitinol **2**, λ_{max} 270 nm [7, 8]). The aromatic ring is evidenced by IR (1585 and 802 cm^{-1}). A doublet at δ6.94 in the ^1H NMR indicates two adjacent aromatic hydrogens at C-6 and C-7. NMR signals show two benzylic methyl groups: one at δ2.15 (Me at C-5) and the other at δ2.25 (Me at C-8). Assignment of a double bond at C-3 was based on IR (1684 cm^{-1}) and ^1H NMR (δ5.73) indicating a vinyl hydrogen.

Identification of the acetate moiety was supported by MS peaks at m/z 216 $[M-CH_2CO]^+$, 199 $[M-MeCO_2]^+$ and 198 $[M-MeCO_2H]^+$. The IR absorptions (in cm^{-1}) at 1733 and 1257 concur with this assignment. The ^1H NMR peak at δ1.97 corresponds to the hydrogens of the acetyl methyl.

The rest of the molecule was elucidated from MS peaks at 201 $[M-CH_2CO-Me]^+$, 184 $[M-CH_3CO_2-Me]^+$, 173 $[M-CH_2CO_2C(CH_3)]^+$, 158 $[M-(CH_3)_2CCO_2CH_3+H]^+$ and 157 $[M-(CH_3)_2CCO_2CH_3]^+$ and IR at 1097 cm^{-1} (C–O and C–C stretch of propyl). The two ^1H NMR peaks at δ1.28 and 1.32 for the methyls at C-11 and C-13 respectively, indicate two isolated methyls.



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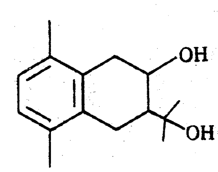

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The TLC staining reaction of **1** (R_f 0.67–see Experimental) was identical to that of another apparent stress metabolite (R_f 0.62.) which had analytical values identical in all respects to rishitinol [7, 8]. The new SSM **1** may be derived from rishitinol (**2**) by dehydration and by acetylation and we therefore propose the trivial name for **1** of acetyldehydrorishitinol.

EXPERIMENTAL

SSMs were extracted from tubers of *Solanum tuberosum* cv. Katahdin stored for 3–6 months postharvest at 10° and treated by the method of Alves *et al.* [5]. A controlled atmosphere of ethylene in oxygen (10 μl ethylene/l. [1% (v/v) N_2; balance O_2]) was used for both the 24 hr pre-incubation period prior to, and the 72 hr incubation period following challenge with the *P. infestans* cell-free extract. SSMs were extracted and purified as previously described [5].

Crude MeOH extracts were streaked across prescored silica gel TLC plates (Analtech, Inc.), and developed in cyclohexane–EtOAc (1:1). The endplates were separated, sprayed with $SbCl_3$-saturated $CHCl_3$, and heated at 110° for 5 min. The band (R_f 0.67, acetyldehydrorishitinol) was eluted with MeOH and purified by HPLC. Analytical columns were used in series: a 30.0 cm × 5.4 mm 10 μm Lichrosorb Si-60 column; and a 30.0 cm × 3.9 mm 10 μm μ-Porasil column. In addition, a 5.0 cm × 4.6 mm precolumn packed with 37–75 μm Porasil A was used. The samples were run with a EtOAc–cyclohexane (2:3) solvent at ambient temperature (flow rate 2.0 ml/min; pressure 1800–3250 psi). Purification by HPLC was monitored by analytical TLC.

Additional chromatographic separation was performed on a silica gel (Silica Woelm TSC for dry column chromatography with 0.5% inorganic fluorescence indicator for short wave UV) column, 3.0 cm × 30.0 cm topped with 3.0 cm of adsorptive magnesia. The column was eluted using a gradient from 100% hexane with increments of 2.0% EtOAc. Acetyldehydrorishitinol was eluted from the column with 10% EtOAc. Purity of the sample was determined by GC [9].

The yield of acetyldehydrorishitinol was *ca* 40 ng per g tuber tissue extracted. λ_{max}^{MeOH} 268 nm; IR ν_{max}^{KBr} cm^{-1}: 2962, 2871, 2925, 2854, 1733, 1684 (C=CH), 1585, 1478, 1378, 1257, 1097, 802. Calc. for $C_{17}H_{22}O_2$: MW, 258.3618. Found: MW (MS), 258.4, 0.7%. Other significant peaks from GC/MS, 70 eV were at m/z: 216, 202, 201, 199, 198, 184, 183, 173, 159, 158, 157, 156, 155, 145. ^1H NMR (250 MHz, $CDCl_3$, TMS as standard): δ 1.28, 1.32, 1.97, 2.15, 2.25, 2.79, 5.73, 6.94.

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